High-level Production of Antibody Derivatives and Alternative Antibody Isotypes Using Scalable Cell Engineering

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Abstract

Antibody derivatives, such as bispecifics and Fc fragments, as well as alternative, non-IgG1 antibody isotypes represent promising classes of cancer immunotherapy. Their expression, however, can be challenging, complicating preclinical evaluation which can require significant amounts of recombinant protein. In this poster we highlight the production of milligram to gram quantities of quality bispecifics and other novel antibody derivatives and isotypes in cells relevant to bioproduction, including multiple CHO cell lines, using MaxCyte's scalable cell engineering technology. We present data showing the high quality of transiently expressed antibody derivatives and their functional characterization. This capacity for rapid, large-scale production of high quality, functional antibody derivatives via transient expression is key to enabling development of novel biotherapeutics with improved efficacy, ADME profiles and manufacturability.

Strong Expression of Her2 x CD3 & CD19 x CD3 Functional BiTEs

Antigen-specific Binding & Redirected Lysis by Non-stimulated αβ T Cells

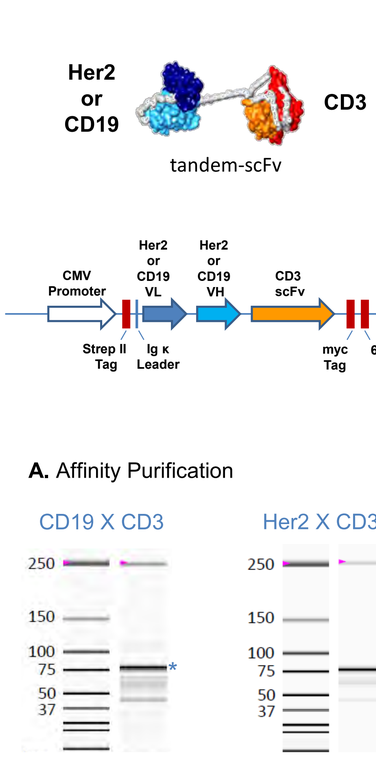


Figure 1: Expression of Functional Bispecific T-cell Engaging (BiTE) Molecules in CHO Cells. 3e9 CHO-S cells were electroporated with a plasmid encoding a Her2 x CD3 or CD19 x CD3 tandem scFv. *A).* Conditioned media samples from electroporated CHO-S cultures were equilibrated in 6xHis binding buffer, and proteins enriched using Ni-NTA agarose. After dialysis against PBS, proteins were assayed by capillary electrophoresis. *B).* BiTE binding to SKBR-3 cells (Breast Cancer, Her2+/CD19-) and Raji cells (Burkitt Lyphoma, her2-/CD19+). *C).* Tumor cells were co-cultured with non-stimulated T-cells for 20 hours in the presence of [$(Her2)_2$ x CD3] or [CD19 x CD3] BiTE molecules and cell lysis measured by a chromium release assay. *Data Courtesy of Dr. Matthias Peipp, Division of Stem Cell Transplantation and Immunotherapy, Christian-Albrechts-University.*

High Expression of Tribody Targeting Her2 x CD16

Augmentation of Antigen-specific, NK-cell-mediated Cytotoxicity

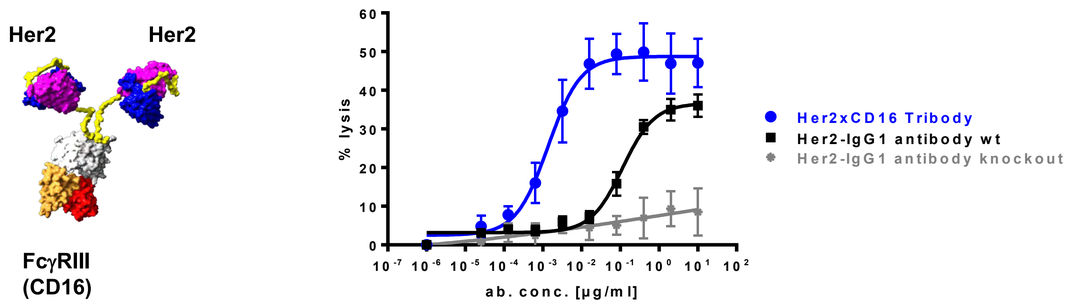


Figure 2: NK-mediated Lysis of Her2+ Tumor Cells Augmented by Tribodies. CHO-S cells were co-transfected with plasmids encoding a Her2 x CD16 tribody. SKBR-3 cells (derived from human breast cancer) were incubated with [$(Her2)_2$ x CD16] tribody or with CD19 x CD3 BiTE molecules. FACS analysis showed binding of the tribody to Her2 antigens on SKBR-3 cells, whereas binding was not observed using a negative control CD19 x CD3 bispecific molecule (data not shown). SKBR-3 cells were co-cultured with mononuclear cells (MNC) at a 40:1 effector to target ratio in the presence of [$(Her2)_2$ x CD16] tribody and cell lysis measured in a 4 hour chromium release assay. *Data Courtesy of Dr. Matthias Peipp, Division of Stem Cell Transplantation and Immunotherapy, Christian-Albrechts-University.*

Improved CHO-S Production of Bispecific Diabody

Higher Quality and Titer Using MaxCyte Electroporation Compared to Lipid Reagents

Transfection Method	Expression (purified protein)	%HMW	%Monomer
MaxCyte STX	**173 .0 mg/L**	5.6	94.3
Lipid Reagent	7.3 mg/L	7.2	92.8

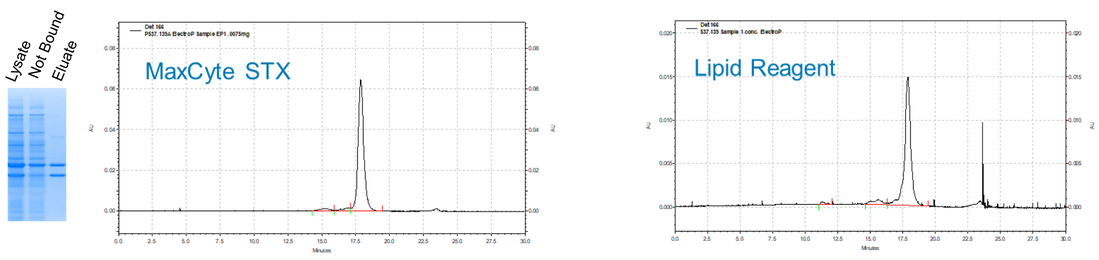
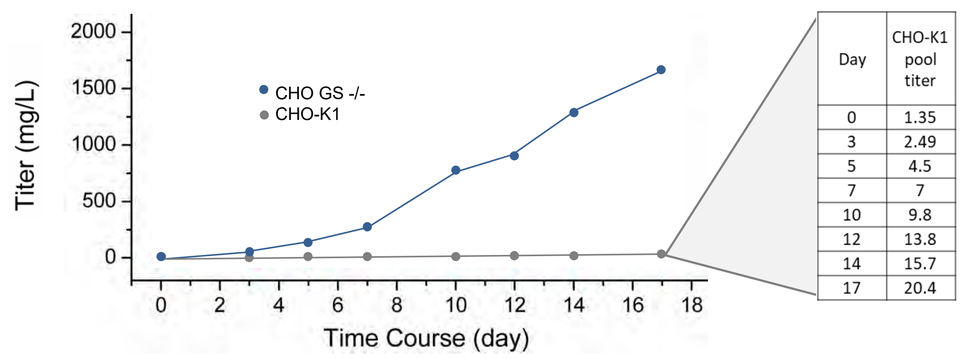


Figure 3: Production of Quality Diabodies. CHO-S cells were transfected with a bicistronic expression plasmid encoding the components of a bispecific diabody via electroporation using the MaxCyte STX or the customer's previous lipid-based transfection reagent. Concentrations of purified diabody were measured using ELISA. Diabody titers were more than 20-fold higher using MaxCyte electroporation. Analysis of purified proteins showed that >94% of the MaxCyte-produced protein was in a monomeric form.

>1.5 g/L Fc Fusion Production

Custom CHO GS -/- Cell Line Stable Pool Outperforms Parental CHO-K1 Cell Line



Day	CHO-K1 pool titer
0	1.35
3	2.49
5	4.5
7	7
10	9.8
12	13.8
14	15.7
17	20.4

Figure 4: Strong Fc Fusion Protein Production From CHO GS -/- Stable Pool. A CHO GS -/- cell line was created from parental CHO-K1 line. Either parental CHO-K1 or CHO GS -/- cells were electroporated with a construct expressing an Fc fusion protein using MaxCyte electroporation. Stable pools were maintained for 17 days. Titers produced by CHO GS -/- stable pools exceeded 1.5 g/L while parental cell line pools produced <25 mg/L. *Data courtesy of LakePharma.*

Expression of Functional CD20-specific IgA2

IgA: Increased Recruitment of Myeloid Effector Cells

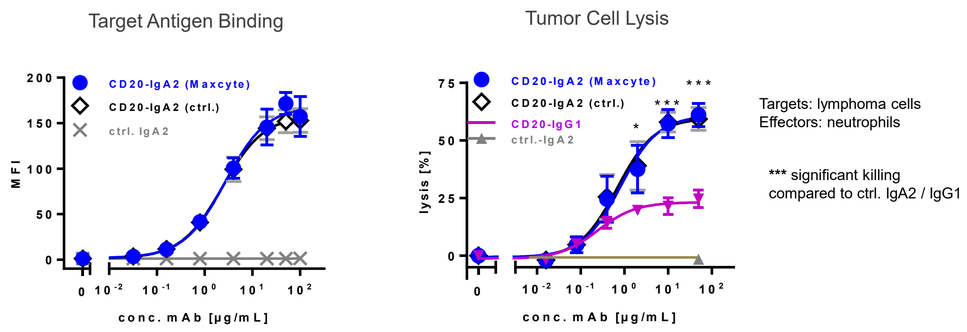


Figure 5: Increased Neutrophil-mediated Tumor Lysis via CD20-specific IgA2. 3e9 CHO-S cells were electroporated using large-scale MaxCyte electroporation with DNA encoding a CD20-specific IgA2. Purified IgA2 was incubated with CD20-bearing lymphoma cells and binding assessed. CD20-specific IgA2 control antibody and non-specific CD20-IgA2, as well as CD20-IgA2 expressed using MaxCyte electroporation were used in a chromium release cytotoxicity assay. *Data Courtesy of Dr. Matthias Peipp, Division of Stem Cell Transplantation and Immunotherapy, Christian-Albrechts-University.*

High Titer IgG4 Antibody Production Using ExpiCHO Cell Line

IgG4 Titers Improved 4-6x by Post Transfection Seed Density Optimization

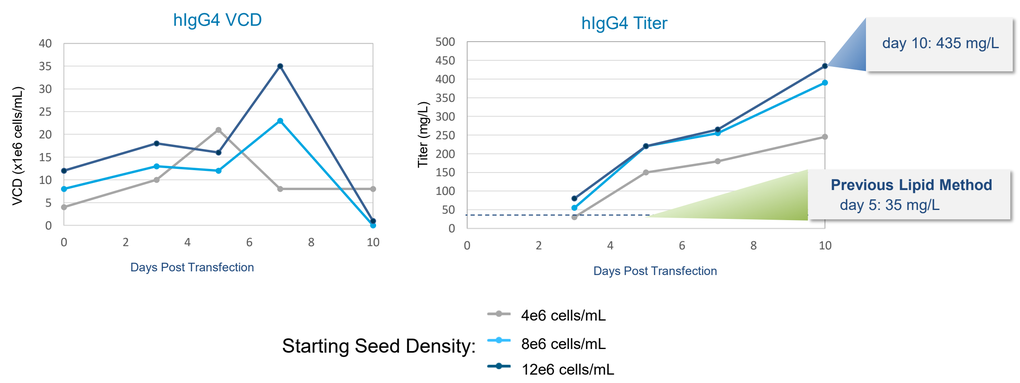


Figure 6: Higher ExpiCHO IgG4 Titers Using Flow Electroporation Technology. ExpiCHO cells were transfected with a human IgG4 expression plasmids using MaxCyte electroporation. Transfected cells were seeded at 4e6 cells/mL, 6e6 cells/mL, or 1.2e7 cells/mL using ExpiCHO medium and feed. Viable cell density and antibody titers were determined at Days 3, 5, 7 and 10 post transfection.

Summary

- Flow Electroporation Technology is universal in nature and can produce a variety of high quality protein types including BiTEs, tribodies, Fc fusion proteins, and alternative antibody isotypes such as IgA2 and IgG4.
- Ability to express alternative antibody-like molecules or non-IgG isotypes allows for fine-tuning of therapeutic effector functions.
- High titer production using a variety of CHO cell lines allow maximum flexibility and early alignment with manufacturing cell line.
- Large-scale MaxCyte electroporation transfects up to 2e10 cells without the need for reoptimization allowing for purification of mg to gram quantities of antibodies from a single transfection.

MaxCyte STX®

5E5 Cells in Seconds
Up to 2E10 Cells in <30 min

- High efficiency transfection with high cell viability
- Broad cell compatibility
- True scalability requiring no re-optimization
- Single cGMP-compliant buffer for all cell types
- Closed, computer-controlled, easy-to-use instrument

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